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Analysis of total meat intake and exposure to individual heterocyclic amines in a case-control study of colorectal cancer: contribution of metabolic variation to risk

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Abstract

A case-control study of colorectal cancer, consisting of 157 cases and 380 controls matched by sex, ethnicity, decade of age and county of residence was performed to explore the associations between environmental exposure, metabolic polymorphisms and cancer risk. Participants were required to provide a blood sample, undergo caffeine phenotyping and complete an in-person interview that evaluated meat consumption, cooking methods and degree of doneness. A color atlas of foods cooked to different degrees of doneness was used to estimate food preparation techniques and food models were used to estimate serving portion sizes. Data was analyzed using a reference database of heterocyclic amine (HCA) exposure based on the food preferences chosen from the atlas. Data regarding individual food items cooked to different levels of doneness, as well as summary variables of foods and of food groups cooked to different degrees of doneness were also evaluated in a univariate analysis for association with colorectal cancer case status. Three measures of metabolic variation, *hGSTA1* genotype, *SULT1A1* genotype and the phenotype for CYP2A6 were also evaluated for possible association with colon cancer.

While higher exposure to HCAs was strongly associated with colorectal cancer risk, increased consumption of five red meats cooked well done or very well done produced comparable odds ratios (OR) for colorectal cancer risk (OR = 4.36, 95% CI 2.08–9.60) for the highest quartile of exposure. Similarly, individuals in the most rapid CYP2A6 phenotype quartile showed an odds ratio (OR = 4.18, 95% CI 2.03–8.90). The ORs for the low activity *hGSTA1* and low activity *SULT1A1* alleles were 2.0, 95% CI 1.0–3.7 and 0.6, 95% CI 0.3–1.1, respectively. Individual measures of specific HCAs provided little improvement in

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risk assessment over the measure of meat consumption, suggesting that exposure to other environmental or dietary carcinogens such as nitrosamines or undefined HCAs may contribute to colorectal cancer risk.
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1. Introduction

Colorectal cancer is the fourth most common cancer worldwide, however, the incidence of this disease is much greater in developed countries than in other parts of the world. Although the results of epidemiological studies are not entirely consistent, components of the Western diet, particularly high fat and meat consumption, are closely associated with the risk of colorectal cancer [1]. In 1983, Nagao et al. [2] demonstrated that specific heterocyclic amines (HCAs) produced by pyrrolysis of meats cooked at high temperatures were highly mutagenic. Since this discovery, the association between meat consumption and colorectal cancer risk has been postulated to involve increased exposure to HCAs due to cooking methods.

Animal studies have demonstrated that exposure to HCAs results in the development of colon tumors in rodents (reviewed in [3]). It is also recognized that metabolic activation of the HCA greatly enhances its mutagenic potency [4–6]. Bioactivation of HCAs to carcinogenic species in vivo is initiated by *N*-oxidation of the compound. This reaction occurs primarily in the liver and, depending on the species, is catalyzed by cytochrome P4501A2 or P4501A1 (CYP1A2 or CYP1A1). Additional metabolism by acetyltransferases (NAT) and sulfotransferases (SULT) generates *N*-acetoxy and *N*-sulfonyloxy esters, electrophiles that are much more reactive with DNA [7,8], resulting in a compound with enhanced mutagenic and carcinogenic potency.

We have proposed a model of colorectal cancer in humans that considers the combination of rapid *N*-oxidation and rapid *O*-acetylation phenotypes along with the level of exposure to HCAs [9]. In a case-control study of colorectal cancer, we evaluated metabolic phenotypes for CYP1A2 and *N*-acetyltransferase 2 in combination with lifestyle variables collected in a patient questionnaire. We found that rapid phenotypes for these enzymes in conjunction with a preference for well-done meat conferred an increased risk of colorectal cancer (OR = 6.45) [9]. Although it was hypothesized that increased intake of well-done meats was responsible for increased exposure to HCAs, the specific characteristics of the meat responsible for this association is not well defined. To address this question, we have conducted a case-control study of colorectal cancer to explore the associations between specific HCA exposures, meat intake and cooking methods, metabolic polymorphisms and cancer risk. Lifestyle and food frequency questionnaires were administered to study participants. Responses to questions of meat intake and cooking methods were analyzed for content of specific HCAs (2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine (PhIP, CAS#105650-23-5), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) CAS# 77500-04-0; 2-amino-4,8-dimethylimidazo[4,5-f]quinoxaline (DiMeIQx) CAS#95896-78-9) using a reference database developed by Sinha and collaborators [10–14].

Three measures of metabolic variation, *SULT1A1* genotype, glutathione *S*-transferase A1 (*hGSTA1*) genotype and cytochrome P450 2A6 (CYP2A6) phenotype were also evaluated for possible association with colorectal cancer. Among the HCAs, PhIP is considered to be important with respect to colorectal cancer risk because it is the most mass-abundant HCA in cooked meats. We have shown that SULT1A1 is capable of catalyzing the sulfation of *N*-hydroxy-PhIP to a reactive sulfonyloxy metabolite that can bind to DNA [8] and the extent of DNA binding is influenced by a genetic polymorphism in the *SULT1A1* gene [15]. Therefore, we examined genotypes for *SULT1A1* in the present study to determine if this genotype was related to risk of colorectal cancer in our study population.

While sulfation and acetylation are activation pathways for HCAs [7,8,16], several detoxification pathways exist, including reduction of reactive electrophilic HCA metabolites with glutathione [17,18]. Human GSTA1 has been shown to be the most effective GST in detoxifying the reactive PhIP metabolite

N-acetoxy-PhIP [19]. We have described a polymorphism in the promoter region of the *hGSTA1* gene that predicts the levels of hepatic expression of both *hGSTA1* and *hGSTA2* and, perhaps more importantly, the ratio of *hGSTA1*/*GSTA2* expression [20]. Since hepatic detoxification of reactive metabolites of HCAs via ester reduction with glutathione could reduce exposure to carcinogenic species in the target tissue (i.e. the colon), we examined the effect of *hGSTA1* genotype on susceptibility to colorectal cancer [19].

In addition to HCAs, recent results from large prospective studies have indicated that both exposure to nitrosamines in the diet and smoking cigarettes are positively associated with the risk of developing colorectal cancer [21,22]. Several studies (reviewed in [23]) have reported increased colorectal cancer risk associated with consumption of processed meats, which are important sources of a variety of nitrosamines. CYP2A6 catalyzes the metabolic activation of a variety of dietary and tobacco-specific nitrosamines; consequently, we hypothesized a role for CYP2A6 in the etiology of colorectal cancer. In this study, CYP2A6 activity, measured by analysis of caffeine metabolites in urine, was positively associated with colorectal cancer incidence [24].

We developed a logistic regression model of colorectal cancer based on these parameters. We included five meats in the model (burgers, steaks, pork chops, bacon and sausage) cooked well or very well done and expressed as quartiles of exposure. HCAs were evaluated individually and as a summary variable and univariate analysis for association with case status was performed. *SULT1A1* and *hGSTA1* genotypes were expressed as dichotomous variables and CYP2A6 phenotype was expressed as quartiles of activity. These studies have expanded our current model of HCA involvement in colorectal cancer etiology to include other pathways of activation and detoxification of these molecules and to include metabolic activation of dietary and tobacco-specific nitrosamines.

2. Materials and methods

2.1. Study population

Participants in this study included those diagnosed with histologically confirmed cancer of the colon or rectum, diagnosed in 1993 through 1999, and community controls. This study population has been previously described [19,24]. Case subjects were recruited from the University of Arkansas for Medical Sciences (UAMS) University Hospital and the Central Arkansas Veteran's Health Care System in Little Rock, Arkansas and from Methodist Hospital, Memphis, Tennessee. Control participants were selected using the Arkansas state driver's license/identity card records and were matched to cases on race, age (within 10 years) and county of residence. The UAMS Institutional Review Board approved the study protocol. We conducted in-person interviews with each subject, obtained a blood sample for genotyping, and asked participants to complete a caffeine phenotyping assay.

2.2. Analysis of exposure to specific HCAs and total meat intake

The interview addressed risk factors for colorectal cancer including cigarette smoking history, occupational history, diet and medical history. Meat consumption was assessed using an instrument, developed by Sinha and Rothman [25], which included detailed questions about meat, fish, and eggs. The data was collected using an in person interview technique, usually performed in the subject's home, with the assistance of a color atlas of foods cooked to different degrees of doneness to estimate food preparation techniques and food models to estimate serving portion sizes. The information was then analyzed using a reference database of HCA exposure (MeIQx, DiMeIQx and PhIP individually and as a summary variable) based on the foods photographed in the above referenced atlas. Data regarding individual food items cooked to different levels of doneness, summary variables of foods and of food groups cooked to different degrees of doneness were also evaluated in a univariate analysis for association with case status. We calculated the amount of each food consumed per week in grams as the product of number of servings per week and serving size. The total amount of meat consumed was estimated by summing the following meat items: hamburger, beef steak, pork chops, ham steak, bacon, sausage, hot dogs, fried chicken, other chicken, turkey, roast beef, beef stew, ground beef, ham, bologna, salami, tomato sauces containing meat,

and meat soups. Categories of meat consumption were established according to the median consumption and quartiles among controls. One hundred and fifty-six cases and 366 controls were interviewed; 24 case subjects and 16 controls did not complete the phenotyping assay, and DNA was not available for every genotyping assay. Omitting subjects for whom no case/control match was available, we reported on caffeine phenotyping results for 127 cases and 333 controls [24]. The *hGSTA1* genotype analysis was performed on 100 colorectal cancer patients and 226 control subjects [19]. DNA was available for few African Americans, therefore, only Caucasians were evaluated for the contribution of *hGSTA1* genotype to colorectal cancer risk. *SULT1A1* genotype was determined for 133 Caucasian colorectal cancer patients and 273 control subjects.

2.3. DNA isolation

A 24 ml blood sample was collected from each participant in four Vacutainer tubes (Becton Dickinson, Fisher Scientific, Houston, TX) containing ACD (ascorbate citrate dextrose) to prevent platelet aggregation. Individual blood cell components were isolated by centrifugation on discontinuous gradients of Histopaque-1077 and Histopaque 1119 (Sigma, St. Louis, MO) as described by Frame et al. [26]. DNA was extracted from lymphocytes using the Wizard genomic DNA isolation kit (Promega, Madison, WI). DNA was quantified using UV spectrophotometry, and its purity was determined by the ratio of its absorbance at 260 nm versus 280 nm.

2.4. SULT1A1 genotype analysis

The restriction endonuclease *Hae*II was purchased from New England Biolabs (Beverly MA). *Taq* DNA polymerase, along with other PCR reagents was purchased from Promega (Madison, WI). Metaphor agarose was obtained from FMC Bioproducts (Rockland, ME). The *SULT1A1* polymorphism under investigation consists of a G to A transition, resulting in an amino acid change (Arg to His, designated *SULT1A1*1* and *SULT1A1*2*, respectively) at amino acid residue 213. Detection of the polymorphism was performed according to the method of Ozawa et al. [27]. Individuals homozygous for *SULT1A1*1* exhibit two bands of 181 and 100 bp upon digestion while PCR products from *SULT1A1*2* homozygotes are not cleaved by HaeII, and a band at 281 bp is observed.

2.5. hGSTA1 genotype analysis

Genotypes for *hGSTA1* were determined as previously described [20]. Briefly, PCR amplification of the region −447 to +33 nucleotides was performed using *Taq* DNA polymerase (Perkin—Elmer, Rockford, IL, USA). The resulting product was exposed to the restriction endonuclease Ear I (New England Biolabs, Beverly, MA, USA) and the digested fragments were resolved using 3% Metaphor agarose gel electrophoresis.

2.6. CYP2A6 caffeine phenotyping

Study subjects were instructed to abstain from methylxanthine-containing foods and beverages (i.e. coffee, tea, chocolate, cola drinks) from midnight before phenotyping until 5 h post-dosing. A 200 mg tablet of No-Doz (Bristol-Myers) was administered to each participant. Four hours post-administration, the subject emptied his or her bladder, and a urine sample representing the 4–5 h urine was collected 1 h later. Urinary caffeine metabolites were extracted and prepared for HPLC analysis as described by Butler et al. [28]. We calculated caffeine metabolite ratios from molar concentrations of caffeine metabolites in the urine samples. Enzyme activity of CYP2A6 was estimated by the ratio of 17U/17X [24].

2.7. Statistical analyses

The distribution of *SULT1A1* genotypes among controls according to demographic characteristics and smoking was examined using contingency tables and Pearson's χ^2 test. Odds ratios (OR) for association between *SULT1A1* genotype and colorectal cancer were calculated initially from a conditional logistic regression model, taking matching into account. Some subjects with genotype data had to be dropped from this analysis due to missing data (no blood drawn) for the matched case or control. We then calculated ORs from an unconditional logistic regression model, including all subjects, with matching factors included

as covariates. ORs for categories of *SULT1A1* genotype were essentially identical. We present ORs and 95% confidence intervals (CI) from the unconditional logistic regression because this analysis allowed us to use all available genotype data. Likelihood ratio tests comparing models with and without a variable representing the number of *SULT1A1*2* alleles (0,1,2) were used to assess trend. ORs for *SULT1A1* genotype within subgroups by age, sex and race were estimated from a logistic model that included interaction terms. Effect modification was tested by likelihood ratio test for the addition of interaction terms to the model. For *hGSTA1*, the distribution of genotypes between cancer patients and control subjects was compared using Pearson's χ^2 test. ORs and 95% confidence intervals were calculated using conditional logistical regression with adjustments for age.

The distribution of the CYP2A6 phenotype among controls was tested for normality using Wilk-Shapiro test. We evaluated relationships between metabolite ratios and subject characteristics (i.e. smoking status, gender, age, and race) among control subjects using Wilcoxon rank-sum test and non-parametric test for trend, and using multivariate linear regression analysis. We evaluated CYP2A6 activity as a risk factor for colorectal cancer using conditional logistic regression analysis of matched case-control pairs, calculating ORs for the lowest, middle and highest quartiles of enzyme activity, with the first quartile as the reference category. A likelihood ratio test for a variable representing the ordered quartiles of activity was used to test for trend. Current smoking and educational background, potential confounders of dietary exposures, were included in the model as covariates.

3. Results

3.1. Study population

The demographics of this study population are shown in Table 1 and have been described elsewhere [19,25]. Briefly, the range of ages for case subjects was 33–87 years (mean 63.4, median 65); for control subjects, the range was 20–88 years (mean 60.8, median 63); 30% of cases and 34% of controls were females. The majority of subjects were Caucasian; 14% of cases and 11% of controls were African American, and none reported other racial background.

3.2. Analysis of individual HCAs and types of meats consumed

The content of individual HCAs was estimated as described by Sinha et al. [29]. Table 2 shows the mean, median and range of MeIQx, PhIP and DiMeIQx, as well as the sum of these parameters for colorectal cancer patients and control subjects. For each HCA examined and for total HCA intake, the cancer patients had consistently higher exposures than did the control subjects. These differences were statistically significant, with p values ranging from 0.0001 for MeIQx to 0.012 for PhIP. When intake of specific meat types was considered in relation to colorectal cancer risk, intake of steak, pork chops, bacon and sausage were all significantly associated with this disease. *P* values for these variables ranged from 0.0001 for bacon to 0.0105 for sausage.

Table 1
Demographics of the study population

	Control subjects ($n = 380$)			Colorectal cancer patients ($n = 155$)		
	Mean	Median	Range	Mean	Median	Range
Age	60.8	63	20–88	63.4	65	33–87
Gender						
Male (*n*)	249			109		
Female (*n*)	131			46		
Ethnicity						
Caucasian (*n*)	333			131		
African–American (*n*)	47			24		

Table 2
Comparison of individual HCA intake and meats consumed between controls and cases

Variable	Control subjects ($n = 380$)			Colorectal cancer patients ($n = 155$)			P[a]
	Mean	Median	Range	Mean	Median	Range	
MeIQx (ng/day)	93.54	53.77	0–993.72	135.38	88.22	0–717	0.0001
PhIP (ng/day)	160.48	89.8	0–2406.9	218.13	130.9	0–3862	0.012
DiMeIQx (ng/day)	6.54	3.2	0–85.4	10.66	5.07	0–138	0.0004
Total HCAs (ng/day)	260.56	162.7	0–3479	364.12	232.4	0–4645	0.0008
Burgers (g/day)	21.57	12.0	0–238	28.57	16.0	0–336	0.079
Steak (g/day)	19.13	10.27	0–264	27.32	18.67	0–252	0.0027
Pork Chops (g/day)	9.45	4.67	0–112	16.46	8.17	0–196	0.0004
Bacon (g/day)	3.7	2.0	0–40	7.49	3.43	0–75	0.0001
Sausage (g/day)	6.88	4.5	0–108	6.88	4.5	0–108	0.0105

[a] P value is from Wilcoxon rank-sum test for comparison of two groups.

3.3. Meat Intake, degree of doneness and exposure to MeIQx

Consumption of burgers, steak, pork chops, bacon and sausage were combined and data was obtained concerning the study participant's preferred degree of doneness for each item. Quartiles of consumption were generated for meat cooked rare, medium, well or very well done (data not shown). Adjustments for decade of age, gender or ethnicity had no effect on the model. The odds ratio (OR) for individuals at the highest quartile of meat intake who preferred their meat cooked well or very well done was 4.36, 95% CI 2.08–9.60. Analysis of the quartiles of exposure to MeIQx demonstrated a highly significant association with colorectal cancer risk. As shown in Table 3, the OR for the highest quartile of exposure was comparable to the OR obtained by examining meat consumption in combination with degree of doneness (4.09, 95% CI 1.94–9.08, compared to 4.36, 95% CI 2.08–9.60).

3.4. Contribution of metabolic variation to risk of colorectal cancer

Three measures of metabolic variation, CYP2A6 phenotype, *SULT1A1* genotype and *hGSTA1* genotype were examined for influence on risk of colorectal cancer. We compared the CYP2A6 phenotype in case subjects to that of controls, based on quartiles in control participants (Table 4). A high proportion of case

Table 3
Association between meat, degree of doneness, MeIQx exposure and colorectal cancer

	Controls, n (%)	Cases, n (%)	OR (95% CI)[a]	P[b]
Quartiles of 5 meats cooked well/very well done (g/day)				
Ref.	109 (29)	25 (16)	1.0	
Low	100 (26)	34 (22)	1.91 (0.85–4.41)	0.118
Medium	91 (24)	42 (27)	2.42 (1.11–5.47)	0.025
High	80 (21)	54 (35)	4.36 (2.08–9.60)	0.0001
Quartiles of MeIQx exposure (ng/day)				
Ref.	102 (27)	29 (19)	1.0	
Low	100 (27)	32 (21)	1.75 (0.78–4.05)	0.177
Medium	92 (25)	40 (26)	2.87 (1.32–6.52	0.008
High	79 (21)	53 (34)	4.09 (1.94–9.08)	0.0002

[a] Adjusted for age, gender and ethnicity.
[b] P value from effect likelihood tests of the nominal logistic fit for case-control.

Table 4
Metabolic variations in relation to colorectal cancer

	Controls, *n* (%)	Cases, *n* (%)	OR[a] (95% CI)	*P*
CYP2A6 Activity				
Ref.	87 (25)	13 (10)	1.0	
Low	87 (25)	29 (22)	1.2 (0.57–2.63)	0.61
Medium	88 (25)	38 (29)	2.14 (1.03–4.52)	0.04
High	88 (25)	53 (40)	4.18 (2.03–8.90)	0.0001
Total *n*	350	133		(*P* trend <0.001)
SULT1A1 genotype				
*SULT1A1*1/*1*	101 (33.6)	48 (36.9)	1.0 (–)	
*SULT1A1*1/*2*	145 (48.2)	67 (51.5)	1.0 (0.6–1.6)	
*SULT1A1*2/*2*	55 (18.3)	15 (11.5)	0.6 (0.3–1.1)	0.22
hGSTA1 genotype[b]				
**A/*A*	79 (35)	37 (37)		
**A/*B*	116 (51)	39 (39)		
**B/*B*	31 (14)	24 (24)	2.0 (1.0–3.7)	(χ^2 *P* value 0.04)
Total *n*	226	100		

[a] Adjusted for smoking status, age, gender and ethnicity.
[b] Coles et al. [19].

subjects (40%) had values in the highest quartile of activity. Elevated OR for colorectal cancer were observed for the third and fourth quartiles, with a trend of increased risk associated with increased CYP2A6 activity (*P* for trend <0.001). Categorization by age, smoking status and amount smoked were examined (data not shown). However, the ORs were very similar from all models, indicating no important confounding by age or smoking history. We also considered whether the 17U/17X ratio varied among cases according to time between a case subject's diagnosis and the collection of the urine sample. Median 17U/17X ratio was very similar for samples collected within 6 months of diagnosis, 7–12 months after diagnosis and for samples collected later than 1 year, indicating little difference by time to sample collection (in a non-parametric test for trend, $P = 0.21$) [24].

The distribution of genotypes for *SULT1A1*1/*1*, *SULT1A1*1/*2* and *SULT1A1*2/*2* in the control group compared to patients with colorectal cancer are shown in Table 4. The *SULT1A1*2/*2* genotype (the low activity variant) was associated with a slightly reduced risk (OR 0.6, 95% CI 0.3–1.1) of colorectal cancer. The direction of the OR suggests reduced risk of colorectal cancer with increasing number of *SULT1A1*2* alleles, but the evidence for a trend was non-significant (test for trend, $P = 0.22$). When subjects were stratified by age, the reduced risk of colorectal cancer associated with the *SULT1A1*2/*2* genotype was limited to subjects older than the median age of 64. There was evidence of effect modification by age ($P = 0.04$). The OR for *SULT1A1*2/*2* among females was further from the null than the corresponding OR among males, but the difference was not statistically significant. When the associations were examined by race, the direction of the ORs was reversed among African–Americans compared to Caucasians. African–American case subjects were more likely to have the *SULT1A1*2/*2* variant allele than African–American control individuals. The tests for interaction indicated a significant ($P = 0.04$) difference by race in the association between *SULT1A1* genotype and colorectal cancer. However, the number of African–Americans in this study is small, and confidence intervals around the ORs in this subgroup were large. There was evidence of heterogeneity by race; therefore, we repeated the analysis by age in a subgroup limited to Caucasian subjects. Results were qualitatively similar to the age-stratified model among all subjects. The ORs among Caucasian subjects ≤63 years were 1.5 (95% CI 0.7–3.3) for *SULT1A1*1/*2* and 0.9 (95% CI 0.3–2.8) for *SULT1A1*2/*2*. Among Caucasian subjects aged 64 years and older, ORs were 0.6 (95% CI 0.3–1.2)

for *SULT1A1*1/*2*, and 0.3 (95% CI 0.1–0.8) for *SULT1A1*2/*2*.

There were also differences in distribution of alleles for *hGSTA1* between colorectal cancer patients and control study subjects. The *hGSTA1*B* allele was less frequent in this study population, with the genotype frequencies essentially the same for males and females [19]. The less common *B/*B allele was over-represented in the colorectal cancer patients compared to the control population (Table 4).

4. Discussion

Epidemiological data suggests that meat consumption plays a role in the etiology of colorectal cancer. HCAs formed during cooking of meat may act as human colon carcinogens. Among epidemiologic studies that assessed method of cooking meats, several studies of colorectal cancer [30,31] or of colorectal adenomatous polyps [32,33] have reported increased risk associated with consumption of meat that was cooked well-done or cooked at high temperatures. Both of these conditions favor the production of HCAs. However, some investigations of colorectal cancer have not found evidence for an association with meat cooking or doneness [34,35]. A large case-control study of colon cancer observed only a non-significant trend in risk with meat doneness, but when preparation method and doneness were taken into account by calculating a mutagen index, there was a significant trend of increased risk with increasing mutagen index [36]. These observations have been supported by the identification of HCA adducts in human colon [37,38]. Consequently, we proposed that assessment of exposure to specific HCAs might be superior to analysis of meat intake and cooking methods in predicting risk of colorectal cancer. When we examined quartiles of exposure to a combination of five meats cooked very well done, and quartiles of exposure for MeIQx, calculated ORs were very similar. Specific measures of HCA exposure provided little improvement over the measure of food preparation, possibly due to the presence of other potential food borne carcinogens. In particular, nitrosamines and other HCAs are also found in cooked meat and their presence is not captured by analysis with the current reference database for HCAs.

Participants in this study also underwent caffeine phenotyping, a procedure used to assess the metabolic activity of several enzymes, including CYP2A6. CYP2A6 contributes to the metabolic activation of dietary and tobacco-specific nitrosamines, which have recently been implicated as risk factors for colorectal cancer [21,22]. We examined the relationship of CYP2A6 phenotype measured by the caffeine phenotyping procedure to risk of colorectal cancer in this study population [24]. In case-control comparisons, subjects in the medium and high quartiles of CYP2A6 activity had an increased risk of colorectal cancer compared to subjects with low activity. We found a strong relationship between CYP2A6 activity, measured by the urinary caffeine metabolite ratio of 17U/17X, and colorectal cancer risk. Although this finding is not a definitive demonstration of the role of nitrosamines in colorectal carcinogenesis, the data are suggestive of this due to the role of CYP2A6 in the activation of procarcinogens found in cigarette smoke and in foods, particularly preserved meats.

N-hydroxylation of HCAs, catalyzed primarily by CYP1A2 in the liver, is the initial step in HCA activation. It has been postulated that the *N*-hydroxy HCAs are then transported in the circulation to the target tissue where further metabolism by UDP-glucuronosyltransferases, *N*-acetyltransferases (NAT) or sulfotransferases (SULT) generates either inactive or reactive metabolites [7,8]. Hepatic acetylation of PhIP generates a metabolite that is stable enough to be transported to target tissues [38,39]. *N*-Acetoxy-PhIP is an activated HCA that is a substrate for GSTs [40]. Additionally, in vitro studies have shown that hGSTA1 is the isoform primarily responsible for detoxification of *N*-acetoxy-PhIP [19]. A promoter region polymorphism that appears to determine the level of expression of hGSTA1 and the ratio of hGSTA1/GSTA2 has been identified in this gene [20]. Since hepatic detoxification could reduce exposure to reactive metabolites in the colon, we examined the relationship of this polymorphism to colorectal cancer risk in this study population. We found a significant over-representation of the homozygous *hGSTA1*B* allele in the colorectal cancer patients [19]; this genotype would confer reduced hepatic levels of hGSTA1. However, the number of cases with DNA available for genotyping was small, and this association should be examined in a larger study population.

Investigations in our laboratory using recombinant allelic variants of *SULT1A1* have shown that this isoform is capable of activating both HCAs and aromatic amines to DNA binding species and this activity is substantially reduced in enzymes encoded by the *SULT1A1*2/2* allelic variant [15]. Therefore, we investigated the influence of *SULT1A1* genotype in this study population. Analysis of the control subjects by age, gender, race, education and smoking habits showed no significant effects of these parameters on *SULT1A1* genotype. Coughtrie et al. have reported a higher proportion of the *SULT1A1*1/*1* genotype is associated with older age [41]. Similar stratification of control subjects by age in the current study showed no effect of age on genotype. The reasons for this difference is unknown, but can probably be attributed to differences in sampling between the two studies. When *SULT1A1* genotype of patients with colorectal cancer were compared to control subjects, risk of colorectal cancer for *SULT1A1*1/*2* subjects was very similar to the reference category, *SULT1A1*1/*1*. Homozygosity for the *SULT1A1**2 allele conferred slightly reduced risk for this disease (Table 4). Since the *2/*2 genotype is infrequent (observed in 20% of Caucasians and 8% of African–American controls in this study), a larger study population would be needed to provide the statistical power to detect a significant OR. It should be noted that genotyping alone usually is not a definitive representation of the in vivo situation. The influence of endogenous and exogenous factors on the regulation of the gene and the activity of the enzyme produced can affect the ultimate phenotype encoded by the genotype. Sulfotransferase activity is inhibited by many compounds found in fruits and vegetables [42]. Therefore, more studies of sufficient power to address both genetic and environmental influences on SULT1A1 and its role in the etiology of colorectal cancer are needed.

The availability of genotyped cases is a limitation of the present case-control study. However, the present data are suggestive of a reduced risk associated with the SULT low activity variant among older subjects, reduced risk associated with the high expression levels of hGSTA1, and increased risk associated with high CYP2A6 caffeine phenotype. Consideration of these associations in larger studies may contribute to our emerging understanding of the complex molecular epidemiology of colorectal cancer.

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